ARS 74-39
JANUARY 1967

HEAT STABILITY OF EGG WHITE PROTEINS UNDER MINIMAL CONDITIONS THAT KILL SALMONELLAE

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

THIS REPORT assembles infarmatian fram researches an the stability to heating and an ather characteristics af the prateins of egg white. The selectian and arganization of infarmatian have been determined by a specific objective--ta find the least damaging heat treatment of egg white that will destray salmanellae.

Three af the seven prateins in egg white are invalved in this prablem. Adequate understanding, hawever, has resulted fram a cansiderable number of research effarts, and the purpase here is ta present the mare pertinent results in a manner that supplies a helpful perspective. Data an heat stability of the prateins are evaluated and related ta the heat stability af salmonellae.

Prepared in
Western Utilization Research and Development Division
Agricultural Research Service
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HEAT STABILITY OF EGG WHITE PROTEINS UNDER MINIMAL CONDITIONS THAT KILL SALMONELLAE[1]

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The practical importance of pasteurization as a food processing method is well recognized. Application to egg white is not readily made because egg white is highly heat sensitive. In fact, prior to our recent work (5)[3] it was not possible to heat egg white to temperatures used to pasteurize whole egg, which of course, contains white. Our investigations showed that lowering the pH of egg white and stabilizing the conalbumin with a metal salt greatly improves heat stability. Whites treated in this way are being pasteurized commercially in the United States.

Further studies and evaluations have been conducted to provide a quantitative picture of the changes that occur in egg white proteins under various conditions that are severe enough to destroy salmonellae. The purpose of this report is to evaluate the relative effects of pH, temperature, and metal salts on ovalbumin, conalbumin, and lysozyme and on salmonellae. These three proteins make up about three-fourths of the total solids of egg white, and two of them, conalbumin and lysozyme, are very unstable when heated in egg white (5, 12). Four other proteins of egg white (ovomucin, globulins G_2 and G_3, and ovomucoid) appear to be rather stable at temperatures of 60° to 62°C. Of these, only ovomucoid is known to be much less stable at pH values above rather than below 8 (10).

Heat Stability of Ovalbumin

When heated, ovalbumin can undergo two marked changes that may influence its performance in food products. One of these is denaturation; the other is the recently discovered conversion to S-ovalbumin (15). S-ovalbumin is more heat stable than ovalbumin and therefore would not be expected to coagulate at the same temperature as ovalbumin during the baking of cakes. It is inferred that the increased heat stability is undesirable, since the use of whites from eggs held under conditions that yield S-ovalbumin gives very poor angel food cakes (11).

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[3]Numbers in parentheses refer to literature cited at end of this report.

We have estimated the times required at temperatures of 57° to 67°C. for 1-percent conversion of ovalbumin to S-ovalbumin and for 1-percent denaturation of ovalbumin when the albumin is at pH 7 and at pH 9. The estimates were made by extrapolation of the data of Lewis (9) for denaturation and of Smith and Back (16) for conversion to S-ovalbumin. First-order reaction constants and temperature coefficient data were available at various pH's. The results obtained by extrapolation (fig. 1) show that ovalbumin is

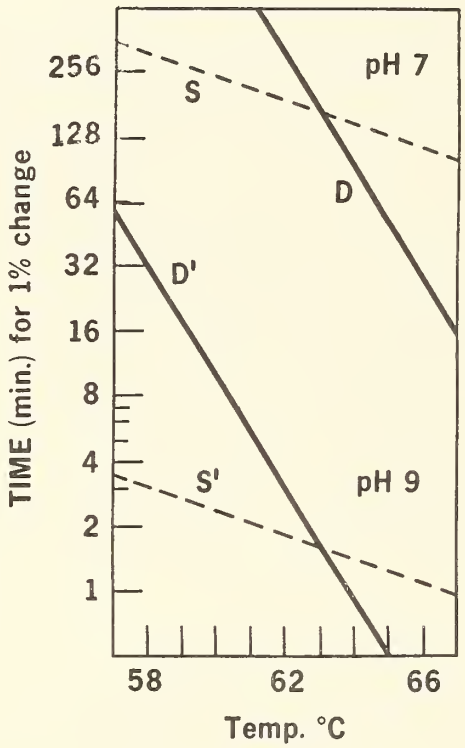

Figure 1. Estimated time in minutes required to change 1 percent of the ovalbumin present in egg white to S-ovalbumin (lines S and S') and to denatured ovalbumin (lines D and D') at temperatures near 60°C. The estimates are extrapolations of the data of Lewis (9) and Smith and Back (16).

about 100 times as heat stable at pH 7 as at pH 9. Since the two deteriorative reactions have different temperature coefficients, the kind of change that will predominate will vary with temperature. Above 63°C. denaturation will be dominant while below 63°C. conversion to S-ovalbumin will be dominant. The data are derived from studies of purified ovalbumin rather than egg white and therefore may not represent exactly the stability of ovalbumin in egg white. However, even if ovalbumin in egg white at pH 7 were five

times as sensitive to heat as these studies indicate, less than 0.1 percent of the ovalbumin would be altered when heated for 3.5 minutes at 60° to 62°C. At pH 9, 1 percent of the ovalbumin would be altered at this time and temperature even if the ovalbumin were only half as sensitive as these studies indicate. Lowering the pH of egg white to pH 7 therefore makes a major improvement in the heat stability of ovalbumin.

Heat Stability of Lysozyme

Lysozyme is remarkably stable to heat in acid solution (4) and is quite stable to heat in mildly alkaline buffer solutions (7). Sandow (14) reported that it is quite unstable to heat in egg white at pH 9. This was confirmed by Cunningham and Lineweaver (5). However, they found that lysozyme in egg white becomes fairly stable near pH 7 when heated at temperatures and for times approximating those used to pasteurize egg products (fig. 2). It was less stable in egg white than in buffer.

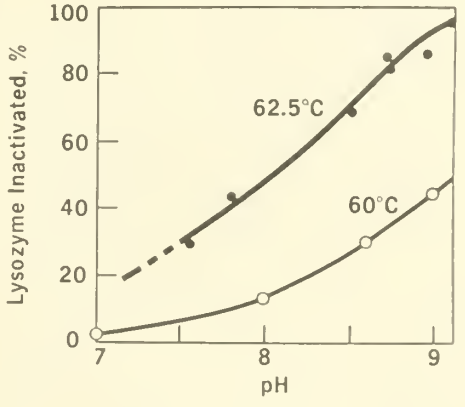

Figure 2. Effect of pH on the heat stability of lysozyme in egg white. Test tubes containing 10 ml. of egg white were held in a water bath for 10 minutes at the indicated temperatures. Samples were analyzed for lytic activity on Micrococcus lysodeikticus cells. The value for pH 7 and 60°C. is barely greater than the error of measurement.

Cunningham (unpublished results) investigated this observation and found that ovalbumin added to a solution of purified lysozyme greatly increases the heat sensitivity of lysozyme. In fact, the destruction of lysozyme occurs at about the same rate in a mixture of purified lysozyme and ovalbumin as in egg white if the mixture contains the same amounts of ovalbumin and lysozyme that occur in egg white. Ovomucoid, ovomucin, and conalbumin added to buffered lysozyme solutions had essentially no effect on the heat stability of lysozyme. The inactivation reaction appears to involve

reduction of one or more of the -S-S bonds in lysozyme by -SH of ovalbumin. This is supported by the finding that the rate of inactivation of lysozyme by heat is markedly increased by cysteine as well as by ovalbumin. The relative rates of inactivation of 2×10^{-4} M lysozyme at pH 9 and 60°C. were: 1 for lysozyme in buffer, 8 for lysozyme in 1.6×10^{-3} M cysteine and 16 for lysozyme in 1.3×10^{-3} M ovalbumin. The starch-gel electrophoretic analysis confirms that lysozyme in egg white is altered markedly at a temperature of 60°C. and pH 9 (fig. 3). Also, "line 18" (fig. 3, area 4), which represents an uncharacterized egg white protein, is absent after heating, and some damage to ovomucoid is evident (fig. 3, area 2).[4]

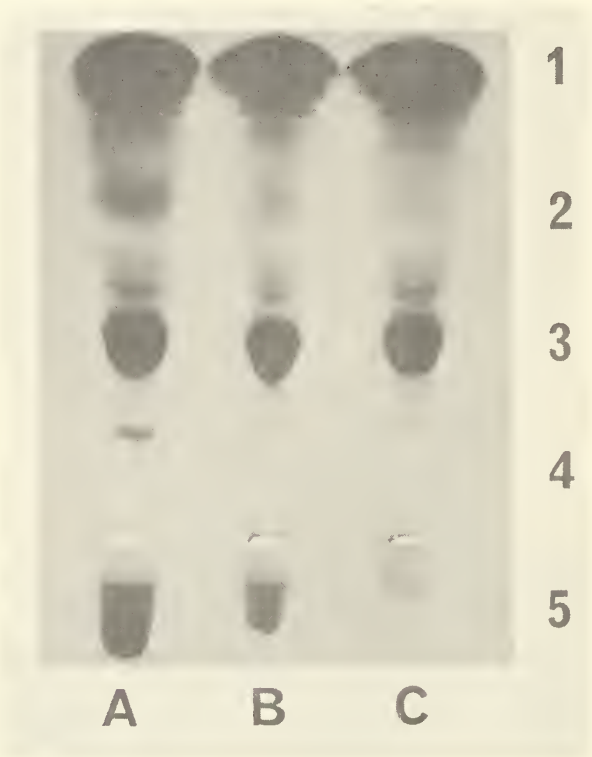

Figure 3. Starch gel electrophoresis patterns of egg white (pH 9) heated in a 60°C. bath. A is the unheated control, B was held in the bath for 5 minutes, and C was held for 10 minutes. Ovalbumin is in area 1, ovomucoid is in area 2, conalbumin is in area 3, "line 18" is in area 4, and lysozyme is in area 5.

[4]Since this paper was presented "line 18" has been characterized by H. T. Miller and R. E. Feeney, Biochemistry 5: 952-958 (1966).

Heat Stability of Conalbumin

Conalbumin was reported to be more heat sensitive than ovalbumin by Osborne and Campbell in 1900 (12). Its stability was found to be least in the pH region 6 to 7 (5) where the stability of ovalbumin is maximum (9). Therefore, neutralizing egg white to stabilize ovalbumin (and lysozyme and ovomucoid) has an undesirable effect on conalbumin. Fortunately, however, the iron-conalbumin complex and certain other metal-conalbumin complexes are stable enough to withstand pasteurization conditions (3, 5). About half of the conalbumin is denatured when egg white at pH 6.8 is heated to 58°C. for 4 to 5 minutes, but the conalbumin-iron complex is not altered at this pH and temperature. That is, it is not precipitated and the red iron-conalbumin color is unaltered in intensity. Similar results are obtained when aluminum is used as the metal. Only about 30 p.p.m. of aluminum ion added to egg white is required for good stability. The aluminum-conalbumin complex is preferred in practice for pasteurization because it is colorless (5). Neither metal ion alters the whipping or other functional property of egg white. The metal ions do not stabilize conalbumin to heat below pH 6 because the complex does not form appreciably on the acid side of pH 6 (17). Therefore, the heat stability of metal-containing egg white is maximum in the range 6.5 to 7.5.

It may be noted that addition of metal ion to whole egg will not improve its heat stability because there is enough iron in the yolk (about 50 p.p.m.) to stabilize the conalbumin of the white.

Conclusions Regarding Heat Stability of the Most Heat-Sensitive Egg White Proteins

From the foregoing evaluation of available data it is estimated that heating pH 9 egg white for 3.5 minutes at 62°C. will alter 3 to 5 percent of the ovalbumin, 90 to 100 percent of the lysozyme and more than 50 percent of the conalbumin. Lowering the pH to 7 reduces the amount of ovalbumin altered by the heat treatment to less than 0.1 percent and the amount of lysozyme altered to less than 6 percent, but increases the amount of conalbumin altered to 100 percent. Formation of the metal conalbumin complex reduces the heat-induced alteration of conalbumin to less than one percent. Lowering the pH and forming the metal salt of conalbumin before heating increases the protein stability of egg white at least 20-fold. That is, the alterations expected in 3-1/2 minutes at 62°C. have been reduced from 10 percent or more of the total protein to less than 0.5 percent.

The viscosity of egg white increases less on heating at pH 7 than at 9 (5). In fact, egg white pasteurized at pH 7 may be thinner than unpasteurized white. This is not due to the heat treatment

but to the pH adjustment and the homogenization that occurs when the white is passed through the heat-exchange equipment.

Pasteurization of egg white may be accompanied by an increase in the whip time even when changes detectable by other means are not evident. Study of the physical-chemical nature of the changes in egg white proteins that accompany the increase in whip time is a challenging problem.

Comparison of the temperature coefficients for damage to egg white and for destruction of salmonellae indicates that a high-temperature short-time process would cause more damage for an equal salmonella kill than the holding procedure now used in the United States (3.5 to 4 minutes at not less than 60°C.). The denaturation of ovalbumin has a temperature coefficient corresponding to an energy of activation of about 130,000 cal. near pH 7 (9). This means that the rate of denaturation will increase about 3 times for each 2°C. increase in temperature. Lysozyme inactivation in egg white also has a high temperature coefficient (energy of activation definitely exceeds 100,000 cal. (fig. 2)), and results thus far obtained indicate that whip time damage occurs about 2.5 to 3 times as fast at 62° as at 60°C.

Salmonella kill, as is true for most vegetative microorganisms, increases about 2 times for a 2°C. increase in temperature (2). Therefore, damage to egg white properties tends to increase faster than salmonella kill as the temperature is raised.

Sensitivity of Salmonellae to Pasteurizing Conditions

We have shown that egg white has maximum stability to heat when it is in the pH region 6.5 to 7.5 provided it contains a metal ion (aluminum preferably) that chelates with and thereby stabilizes conalbumin. Salmonellae are also more stable to heat at pH 7 than at pH 9 (1, 13). However, the stability of the egg white is increased more than the heat resistance of the salmonellae by lowering the pH.

Decimal reduction times (D) for salmonellae at 60°C. calculated from the data of Osborne and others (13) is about 0.1 minute at pH 9 and is 0.4 to 0.5 minute at pH 7 (fig. 4). These results considered alone indicate that pasteurization at pH 9 would be desirable as suggested by Anellis and others (1), Osborne and others (13), and Elleman (6). However, such a course is offset by the damage caused to egg white heated at pH 9 and the finding that a temperature of 60°C. cannot be used even for holds of 5 to 10 seconds at pH 9 (8). A temperature of 56.7°C. at pH 9 would give a D value of about 0.45, which is equal to that at 60°C. and pH 7 but is greater than D values at 61° to 62°C.

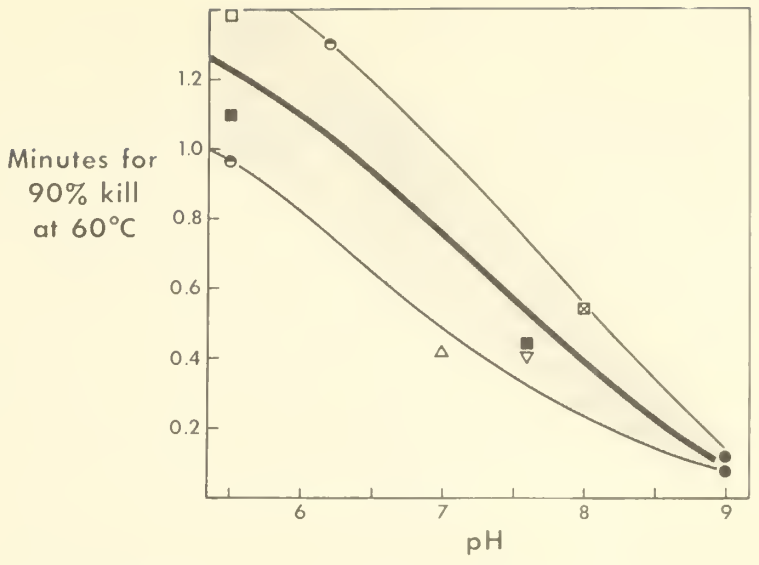

Figure 4. Sensitivity of salmonella to heat at various pH values as indicated by decimal reduction times at 60°C. in egg material. Data in whole egg by Anellis and others (1), □ montevideo, ⊠ both montevideo and typhimurium, ■ worthington. Data of Osborne and others (13), ● several species in egg white (calculated from F_{140} values), ◓ meleagridis in whole egg and yolk, ▽ several species in whole egg. △ Mixture of typhimurium, derby, oranienburg, and worthington in egg white.

The stability of egg white is increased enough by pH adjustment and aluminum addition to permit the use of 61° to 62°C. The decimal reduction time at 61° to 62°C. is about 0.2 minute. Such a D value will give an expected salmonella kill of 10^5 per minute of hold. Even if the D value of a particular salmonella were twice the usual value, a 3.5-minute hold at 61° to 62°C. would give a kill of nearly 10^9.

The data in figure 4 indicate that the heat stability of salmonellae is greater in the pH region 5.5 to 6.5 than it is at pH 7. However, the data are inadequate to thoroughly establish the kill efficiency under the various conditions of pH and additives encountered in the egg industry, and further studies are needed.

The degree of protection provided by pasteurization is of course related to the number of salmonellae present in the untreated liquid. Many samples of eggs have less than one salmonella per gram, and good-quality raw liquid should contain less than a few thousand. Therefore, pasteurization at 60° to 62°C. for 3 to 4 minutes provides a wide margin of safety. That is, it should

reduce the count to less than one in a million grams even if the initial count were 1,000 per gram.

The 775-W strain of Salmonella senftenberg is about 10 times as heat resistant as other strains and serotypes tested. The foregoing comments do not apply to this strain. However, so far as is known, this strain has not been isolated from a natural product since it was first isolated in 1947. We believe the quality of pasteurized products should not be sacrificed to take care of this, as yet at least, nonrecurring strain. Studies of several hundred strains of salmonella are under way in this laboratory to determine whether any are as heat resistant as S. senftenberg 775-W.

These considerations show why egg white, appropriately stabilized, can be effectively pasteurized at 60° to 62°C. in 3 to 4 minutes. Additional information is needed to define effective pasteurization conditions that can be used without serious damage to egg liquid containing added sugar, salt, or other substances.

The relative heat stability of egg white proteins and salmonellae under a variety of conditions has been further evaluated. Salmonellae are more easily destroyed at alkaline pH's (e.g., 9 to 10) than at neutrality. However, lysozyme is rapidly inactivated at pH 9 and ovalbumin is converted to a heat-stable form which produces a poor angel food cake. The inactivation of lysozyme at pH 9 and 60°C. is due to a reaction with ovalbumin, although ovalbumin itself is not appreciably denatured. Comparisons of the damage at various pH values show that only slight damage occurs when egg white is heated to temperatures as high as 62°C. at pH 6.5 to 7.0 in the presence of a metal (e.g., aluminum) that stabilizes the protein conalbumin. In contrast, marked changes occur when unmodified egg white at pH 9 and above is heated above 57°C. These include loss of lysozyme activity, changes in at least two egg white proteins as detected on starch gel electrophoresis patterns, increase in viscosity, and decrease in foaming power. Neutralizing egg white to pH 7.0 and adding a metal salt to stabilize the conalbumin decreases the damage resulting from heat treatment at 60° to 62°C. more than it affects the killing efficiency. The high temperature coefficient of damage indicates that a high-temperature, short-time process would not be an especially effective pasteurization treatment.

LITERATURE CITED

(1) Anellis, A., Lubas, J., and Rayman, M. M.
1954. Heat resistance in liquid eggs of some strains of the genus Salmonella. Food Res. 19: 377-395.

(2) Angelotti, R., Foter, M. J., and Lewis, K. H.
1961. Time-temperature effects on salmonellae and staphylococci in foods. III. Thermal death time studies. Applied Microbiol. 9: 308-315.

(3) Azari, P. R., and Feeney, R. E.
1958. Resistance of metal complexes of conalbumin and transferrin to proteolysis and to thermal denaturation. J. Biol. Chem. 232: 293-302.

(4) Beychok, S., and Warner, R. C.
1959. Denaturation and electrophoretic behavior of lysozyme. J. Amer. Chem. Soc. 81: 1892-1897.

(5) Cunningham, F. E., and Lineweaver, H.
1965. Stabilization of egg white proteins to pasteurizing temperatures above 60°C. Food Technol. 19: 1442-1447.

(6) Elleman, G.
1959. Salmonella infection of eggs. Internatl. Vet. Congr., Madrid, Rpt. 16, pp. 5-7.

(7) Gorni, L., and Felix, F.
1953. Influence of manganese on the stability of lysozyme. I. Influence of manganese on the velocity of irreversible inactivation of lysozyme by heat. Biochim. Biophys. Acta 10: 128-135.

(8) Kline, L., Sugihara, T. F., Bean, M. L., and Ijichi, K.
1965. Heat pasteurization of raw liquid egg white. Food Technol. 19: 1709-1718.

(9) Lewis, P. S.
1926. The kinetics of protein denaturation. II. The effect of variation in the hydrogen ion concentration on the velocity of heat denaturation of egg albumin; the critical increment of the process. Biochem. J. 20: 978-983.

(10) Lineweaver, H., and Murray, C. W.
1947. Identification of the trypsin inhibitor of egg white with ovomucoid. J. Biol. Chem. 171: 565-581.

(11) Meehan, J. J., Sugihara, T. F., and Kline, L.
1962. Relationships between shell egg handling factors and egg product properties. Poultry Sci. 41: 892-900.

(12) Osborne, T. B., and Campbell, G. F.
1900. The protein constituents of egg white. J. Amer. Chem. Soc. 22: 422-450.

(13) Osborne, W. W., Straka, R. P., and Lineweaver, H.
1954. Heat resistance of strains of salmonella in liquid whole egg, egg yolk, and egg white. Food Res. 19: 451-463.

(14) Sandow, A.
1926. The anti-bacterial activity of egg-white. Soc. Expt. Biol. Med. Proc. 24: 172-175.

(15) Smith, M. B.
1964. Studies on ovalbumin. I. Denaturation by heat, and the heterogeneity of ovalbumin. Austral. J. Biol. Sci. 17: 261-270.

(16) _____ and Back, Joan F.
1965. Studies on ovalbumin. II. The formation and properties of S-ovalbumin, a more stable form of ovalbumin. Austral. J. Biol. Sci. 18: 365-377.

(17) Warner, R. C., and Weber, Ione.
1953. The metal combining properties of conalbumin. J. Amer. Chem. Soc. 75: 5094-5101.

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